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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All instrument-generated data were collected using the manufacturer's associated software as described in the Online Methods. Plate reader data were collected by SoftMax Pro (Ver. 5, Molecular Devices). Microarray data were collected by GenePix (Ver. 5.0, Molecular Devices). SPR data were collected by Biacore T200 Evaluation Software (Ver. 1, GE Healthcare). Cell and tissue images were collected by ZEN Software (Ver. 8.1, Carl Zeiss). Gel images were collected by ImageStudio (Ver. 5.2.5, LI-COR Biosciences).
Data analysis	Computational docking studies were conducted using methods and software from a previously described publication (Griffith AR et al. Proc. Natl. Acad. Sci. U.S.A. 114 (2017), 13697-13702). Software associated with this procedure includes SWISS-MODEL for homology model generation (accessed May 2016, https://swissmodel.expasy.org/), DREIDING for minimalization (in house, Ver. May 2016), MPsim for molecular dynamics (in house, Ver. May 2016), and SCREAM for side chain optimization (in house, Ver. May 2016). Imaging data were analyzed using Fiji/ImageJ (Ver. 2.0.0-rc-43/1.51w, NIH). Immunoblotting data were analyzed by ImageStudio (Ver. 5.2.5, LI-COR Biosciences). Guide RNAs were designed by CHOPCHOP (Ver. 2, https://chopchop.cbu.uib.no/). All statistical analyses were conducted by Prism 7 for Mac (Ver. 7.0d, Graphpad) and Microsoft Excel for Mac (Ver. 16.16.2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Any data generated or analyzed during this study are included in the article and related supplemental information or are available from the corresponding author

on reasonable request. Publicly available data used in this study include the Tie2 crystal structure (PDB ID 2GY5), the Tie1 protein sequence (UniProt ID P335590), the Dec. 2011 murine genome assembly (GRCm38/mm10), and the CHOPCHOP guide RNA design tool (https://chopchop.cbu.uib.no/).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical analyses were used to predetermine sample size. Sample sizes for all experiments were chosen based on previous publications from our laboratory (Shipp EL, Hsieh-Wilson LC. Chem. Biol. 14 (2007), 195-208; Brown JM et al. Proc. Natl. Acad. Sci. U.S.A. 109 (2012), 4768-4773; Pulsipher A et al. Angew. Chem. Int. Ed. 54 (2015), 1466-1470) and others (Savant S et al. Cell Rep. 12 (2015): 1761-1773; Korhonen EA et al. J. Clin. Invest. 126 (2016), 3495-3510; Xu D et al. J. Biol. Chem. 286 (2015): 737-745) as cited in the text to account for intrinsic variability of the experiments.
Data exclusions	No data were excluded from analyses.
Replication	All cell-based assays were successfully replicated as independent experiments described in the text and supporting information. All animal tissue-based assays were performed using samples acquired from litter mates from at least two biologically independent litters that were processed and analyzed together after genotyping.
Randomization	No randomization was applied in these experiments. Specific controls for covariates in the protein- and cell-based assays were not relevant as identical protocols and reagents were used within each assay across all samples except for the variable being tested. Animals were allocated based on genotype, and age-matched litter mates were used to minimize covariance in the biological source material.
Blinding	No blinding was applied in these experiments. All data acquisition and analysis were performed automatically where possible by software described above and in the text and supporting information. For the FoxP3 nuclear export assay, the FoxP3 fluorescence signal of the costained nucleus and adjacent cytosol of each cell in a field of view was measured by software, and the categorical, binary assignment of FoxP3 nuclear status of each cell was conducted by a single, unblinded investigator based on this quantitative data to prevent investigator bias.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies used in the manuscript as are follows: Rb anti-His-tag (Cell Signaling Technology, 12698S, D3I1O); Gt anti-Tie1 (R&D Systems, AF619); Rb anti-Tie1 (Cell Signaling Technology, 23111S); Ms anti-Tie1 (R&D Systems, MAB619); Rb anti-phospho-Tie2 (R&D Systems, AF2720); Gt anti-Tie2 (R&D Systems, AF313); Gt anti-Tie2 (R&D Systems, AF762); Rb anti-Tie2 (Santa Cruz Biotechnology, H176); Rb anti-phospho-Akt (Cell Signaling Technology, 9275S); Ms anti-Akt (Cell Signaling Technology, 2920S, 40D4); Ms anti-alpha-tubulin (MilliporeSigma, T9026, DM1A); Rb anti-phospho-p44/42 MAPK (or Erk1/2) (Cell Signaling Technology, 9101S); Ms anti-p44/42 MAPK (or Erk1/2) (Cell Signaling Technology, 4696S, L34F12); Rb anti-FOXO1 (Cell Signaling Technology, 2880S, C29H4); Ms anti-HS (370255-S, Amsbio, F58-10E4); Gt anti-Ms Alexa Fluor 488 (ThermoFisher, A11001); Gt anti-Ms Alexa Fluor 680 (ThermoFisher, A21057); Gt anti-Ms Alexa Fluor 790 (ThermoFisher, A11357), Gt anti-Rb Alexa Fluor 568 (ThermoFisher, A11036), Gt anti-Rb Alexa Fluor 680 (ThermoFisher, A21076), Gt anti-Rb Alexa Fluor 790 (ThermoFisher, A11369), Dk anti-Gt Alexa Fluor 680 (ThermoFisher, A32860), Gt anti-Hm Fc Alexa Fluor 647 (Jackson ImmunoResearch, 109-605-098). Specific lot numbers are unavailable.

Validation	All antibodies were commercially available and used for applications previously validated by the manufacturers and in relevant citations on the manufacturers' websites or in house. Rb anti-His-tag (Cell Signaling Technology, 12698S, D3I1O): Manufacturer validated Western blotting against recombinant protein. Gt anti-Tie1 (R&D Systems, AF619): Manufacturer validated Western blotting using recombinant Tie1. Rb anti-Tie1 (Cell Signaling Technology, 23111S): Manufacturer validated for Western blotting with expressing cell lines. Ms anti-Tie1 (R&D Systems, MAB619): Manufacturer validated for cell imaging and Western blotting using cell lines and recombinant Tie1, respectively. Rb anti-phospho-Tie2 (R&D Systems, AF2720): Manufacturer validated for Western blotting using stimulated cell lines. Gt anti-Tie2 (R&D Systems, AF313): Manufacturer validated for Western blotted using cell lines. Gt anti-Tie2 (R&D Systems, AF762): Manufacturer validated for Western blotting using recombinant protein. Rb anti-Tie2 (Santa Cruz Biotechnology, H176): Manufacturer validated for cell staining using cell lines. Rb anti-phospho-Akt (Cell Signaling Technology, 9275S): Manufacturer validated for Western blotting using stimulated cell lines. Ms anti-Akt (Cell Signaling Technology, 2920S, 40D4): Manufacturer validated for Western blotting using cell lines. Ms anti-alpha-tubulin (MilliporeSigma, T9026, DM1A): Manufacturer validated for Western blotting using cell lines. Rb anti-phospho-p44/42 MAPK (or Erk1/2) (Cell Signaling Technology, 9101S): Manufacturer validated for Western blotting using stimulated cell lines. Ms anti-p44/42 MAPK (or Erk1/2) (Cell Signaling Technology, 4696S, L34F12): Manufacturer validated for Western blotting using cell lines. Rb anti-FOXO1 (Cell Signaling Technology, 2880S, C29H4): Manufacturer validated for cell imaging using stimulated cell lines. Ms anti-HS (370255-S, Amsbio, F58-10E4): Manufacturer validated for cell imaging using knockout cell lines. Gt anti-Ms Alexa Fluor 488 (ThermoFisher, A11001): Manufacturer validated for cell imaging detection using Ms IgG. Gt anti-Ms Alexa Fluor 680 (ThermoFisher, A21057): Manufacturer validated for Western secondary detection using Ms IgG. Gt anti-Ms Alexa Fluor 790 (ThermoFisher, A11357): Manufacturer validated for Western secondary detection using Ms IgG. Gt anti-Rb Alexa Fluor 568 (ThermoFisher, A11036): Manufacturer validated for cell imaging detection using Rb IgG. Gt anti-Rb Alexa Fluor 680 (ThermoFisher, A21076): Manufacturer validated for Western secondary detection using Rb IgG. Gt anti-Rb Alexa Fluor 790 (ThermoFisher, A11369): Manufacturer validated for Western secondary detection using Rb IgG. Dk anti-Gt Alexa Fluor 680 (ThermoFisher, A32860): Manufacturer validated for Western secondary detection using Gt IgG. Gt anti-Hm Fc Alexa Fluor 647 (Jackson ImmunoResearch, 109-605-098): Manufacturer validated for Hm Fc specificity by ELISA and was also co-validated for specificity on microarrays in house with similar results to other Gt anti-Hm Fc secondary antibodies used previously (Brown JM et al. Proc. Natl. Acad. Sci. U.S.A. 109 (2012): 4768-4773).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK-293T (ATCC CRL-3216), EA.hy926 (ATCC CRL-2922), and HUVEC, pooled (ATCC PCS-100-013)
Authentication	Cells were used as received from ATCC without further authentication.
Mycoplasma contamination	Cells were used as received from ATCC without further testing for mycoplasma contamination. No nonnuclear puncta were observed from DAPI staining during immunofluorescence experiments.
Commonly misidentified lines (See ICLAC register)	The study did not use any commonly misidentified cell lines.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	The Tie1-2A mouse line was generated using B6SJL-F1/J founders (Jackson Laboratory) and backcrossed with C57BL/6J mice (Jackson Laboratory) as described in the Online Methods. Animals were maintained with no more than five adult animals per cage in small mouse cages with enrichment under 20-26C ambient temperature, 30-70% relative humidity, and a 12-h light-dark cycle. General animal maintenance and timed matings were performed by members of the Caltech Office of Laboratory Animal Resources with cage changes performed weekly. For retinal tissue harvesting, litter mates of both sexes at P7 were used. For lung tissue harvesting, litter mates of both sexes at 4 months of age were used.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All procedures involving animals were approved by the Caltech Institutional Animal Care and Use Committee prior to the start of all experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.